



Research Article

PRECURSORY PHYSICO-CHEMICAL, INSTRUMENTAL EVALUATION AND ACUTE ORAL TOXICITY STUDY OF *PURANDARA VATI*

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ABSTRACT

Purandara vati is a herbomineral formulation that has a promising effect on *Shwasa*, *Kasa*, and is nothing less than a *Rasayana*. The main ingredients are *Parada*, *Gandhaka*, *Triphala*, and *Trikatu* which is given *Bhavana* in *Aja kshira*. The current paper aims to evaluate the physicochemical parameter along with instrumental analysis followed by an acute oral toxicity study of *Purandara vati*. **Materials and methods:** Physicochemical, instrumental analysis of *Purandara vati* were conducted. An acute oral toxicity study was also conducted as per OECD 425 guidelines. The IAEC no of the study is FPS/IAEC/169/2024. The study was conducted with a total of 4 healthy female Wistar albino rats with 8–10-week-old (150–180g body weight). **Results:** *Purandara vati* has shown a pH of 4.13, loss on drying 9.50%, total ash 4.32%, acid insoluble ash 2.36%, water soluble ash 2.66%. In XRD *Purandara vati* had showed mercury sulphide in the major phase. FTIR analysis identified various functional groups i.e., carboxylic acid, alkane, ester etc. Acute oral toxicity studies showed no mortality at a dose of 2000mg/kg body weight proving the fact that LD50 of *Purandara vati* is above 2000mg/kg. **Conclusion:** *Purandara vati* is a safe and potent herbomineral formulation.

INTRODUCTION

In *Rasashastra*, *Parada* (mercury) is given much importance because, when purified and used correctly, it is considered nothing short of an elixir. The mercurial medicines are administered in smaller doses but have a wide range of therapeutic activity and they have a very fast assimilation rate.^[1] *Rasa yogas* mentioned in classics are classified into four: *Kharaliya*, *Pottali*, *Parpati*, and *Kupipakva Rasayana*. *Kharaliya Rasayana* is *Murcchita yoga* where *Murcchana* (mercurial process) imbibes therapeutic properties to the drug. In this *Yoga* mercury is grounded along with other drugs in a *Khalva*.^[2]


Purandara vati is *Sagandha*, *Niragni murcchita yoga*, explained in the *Kasa chikitsa* of *Rasachandamshu*. It is prepared by triturating *Dwiguna kajjali* (prepared from *Hingulotha Parada* and *Gandhaka* in the ratio 1:2) along with *Trikatu*, *Triphala*

and given *Bhavana* in *Ajakshira*.^[3] It is a potent herbomineral formulation that has *Yogavahi* properties and is said to be beneficial in treating *Shwasa* and *Kasa*. Along with the physicochemical analysis of *Purandara vati*, acute oral toxicity was also performed as per OECD 425 guidelines. In Acute oral toxicity the drug is tested through an up-and-down procedure where the immediate toxicity effect of the drug is monitored. The main test consists of a single-ordered dose progression in which animals are dosed, one at a time, at a minimum of 48-hour intervals. The first animal receives a dose a step below the level of the best estimate of the LD50. If the animal survives, the dose for the next animal is increased by a factor of 3.2 times the original dose; if it dies, the dose for the next animal is decreased by a similar dose progression^[4].

MATERIALS AND METHODS

This study was done to identify the physicochemical properties, crystallographic identification and acute oral toxicity profile of *Purandara vati*.

Method of Preparation: The preparation of *Purandara vati* was done in accordance with *Rasachandamshu* and *Vati* preparation was done as per

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the classical reference. *Hingulotha Parada nirmana*, *Gandhaka shodhana*, *Parada shodhana*, *Purandara vati nirmana* etc, was done in Department of PG and PhD Studies in *Rasashastra* and *Bhaishajya Kalpana* Government Ayurveda Medical College, Bengaluru.

Authentication of herbal drugs used in the preparation was done by the Head, Department of *Dravyaguna Vinjana* Government Ayurveda Medical College Bengaluru.

Table 1: Ingredients of Purnadara vati

S.No	Drug	Chemical/Botanical Name	Part Used	Quantity of Drug
1.	<i>Parada</i>	Mercury		1 Part
2.	<i>Gandhaka</i>	Sulphur		2 Parts
3.	<i>Shunti</i>	<i>Zingiber officinale</i> Family: <i>Zingiberaceae</i>	Rhizome	1 Part
4.	<i>Maricha</i>	<i>Piper nigrum</i> Family: <i>Piperaceae</i>	Fruit	1 Part
5.	<i>Pippali</i>	<i>Piper longum</i> Family: <i>Piperaceae</i>	Fruit	1 Part
6.	<i>Haritaki</i>	<i>Terminalia chebula</i> Family: <i>Combretaceae</i>	Fruit	1 Part
7.	<i>Bibhitaka</i>	<i>Terminalia belerica</i> Family: <i>Combretaceae</i>	Fruit	1 Part
8.	<i>Amalaki</i>	<i>Emblica officinalis</i> Family: <i>Euphorbiaceae</i>	Fruit	1 Part
9.	<i>Ajakshira</i>	Goat's milk		Q. s

Determination of Physico-Chemical Analysis

Physico-chemical analysis of *Purandara vati* was carried out at Government of Karnataka, Drug Testing Lab for Ayush drugs, Jayanagar, Bangalore. Instrumental Analysis such as XRD, SEM-EDS & FT-IR of *Purandara vati* was conducted at IISC, Bangalore.

Determination of pH^[5]

1gm of *Purandara vati* was taken and after calibration of the instrument, 1% aqueous solution of sample was prepared separately and pH was determined for the solution, reading was noted.

Determination of Loss on Drying at 105°C^[6]

- 3 gm of *Purandara vati* was weighed accurately, taken in a tarred dried petri dish. This petri dish is then placed in a preheated hot air oven and dried at 105°C for 5hrs
- Later the dish was taken out and cooled in a desiccator and weighed.

Determination of Total Ash^[6]

2gm of *Purandara vati* was taken in a cleaned, dried, and weighed silica crucible. The crucible is incinerated over the electric stove for 10 min. Later the crucible is placed in a muffle furnace and ignited at temperature not exceeding 600°C until the sample is

free from carbon. Cool and weigh the sample. Calculate the percentage of ash concerning the air-dried drug.

Determination of Acid Insoluble Ash^[5]

The total ash obtained as above is taken in a 250ml beaker and boiled with 25ml of diluted HCl. The mixture was filtered using Ashless Whatman filter paper No. 1. The filter paper along with the sample is kept in a crucible and incinerated in the stove for 10 minutes. Later the crucible is kept in a Muffle furnace at a temperature of 550°C for 5 hrs. The crucible was taken out and allowed to cool in a desiccator and weighed. Calculate the percentage of acid insoluble ash concerning the air-dried drug.

Determination of Water-Soluble Ash^[6]

Ash of the *Purandara vati* is mixed with 25ml of water and poured into a beaker. Then it is filtered through an ashless filter paper. Filter paper along with filtrate is kept in a crucible which is ignited in a stove for 10 min. Later the crucible is kept in Muffle furnace for 5hrs. After cooling weight is noted. Calculate the percentage of water-soluble ash concerning the air-dried drug.

Table 2: Results of Physico chemical analysis

Parameters	Results
pH	4.13
Loss on Drying	9.50%
Total Ash	4.32%
Acid Insoluble Ash	2.36%
Water Soluble Ash	2.66%

Instrumental Analysis**XRD Analysis of Purandara Vati****Table 3: 2 θ value and D space of Purandara vati**

Peak No	Standard			Identified	
	Angle 2 θ	D space	Intensity	Angle 2 θ	D space
179	26.27	3.390	100	26.42	3.307
183	30.42	2.936	28.9	30.54	2.924
192	43.70	2.070	39.3	43.74	2.0681
Name of Standard: Mercury Sulphide Crystal System: Cubic ICDD No: 00-089-0432					

- 3 strong peaks were chosen as strong with their relative intensity and compared to standard X-ray powder diffraction file (XPDF)
- 179th peak with a relative intensity of 100% was significant at angle 26.27 having a 3.390 d space value.
- 8 peaks of mercury sulphide were identified.

SEM EDX Analysis of Purandara vati

Particle size of *Purandara vati* ranges from 26.00nm to 101.320nm.

Elemental Analysis of Purandara vati**Table 4: Results of elemental analysis**

Element	Weight %	Atomic %
C K	55.16	68.04
O K	30.56	28.30
S K	5.52	2.55
K K	1.20	0.46
Ca K	0.34	0.13
Hg M	7.21	0.53

FTIR Analysis of Purandara vati**Table 5: Results of FTIR analysis**

Sample peak frequency cm ⁻¹	Standard peak frequency cm ⁻¹	Specific type of Bond	Bond	Functional group
3273.77	3330-3250	Medium	N-H	Aliphatic primary amine
2923.26	3300-2500	Strong	O-H	Carboxylic acid
2853.25	3000-2800	Strong	N-H	Amine salt
1697.36	1710-1680	Strong	C=O	Conjugated acid
1613.87	1620-1610	Strong	C=C	α,β unsaturated ketone
1446.74	1450-1375	Medium	C-H	Alkane

1332.37	1350-1300	Strong	S=O	Sulphone
1206.62	1210-1163	Strong	C-O	Ester
1152.80	1250-1020	Medium	C-N	Amine
1075.12	1085- 1050	Strong	C-O	Primary alcohol
1015.86	1400-1000	Strong	C-F	Fluorocompound
860.57	880 ± 20	Strong	C-H	1,2,4 tri-substituted benzene derivative

Acute Oral Toxicity Study

Acute Oral toxicity study of *Purandara vati* was carried out at Dept. of Pharmacology, Faculty of pharmaceutical science, PES University Electronic City, Bengaluru, Karnataka, CPCSEA Reg: 600/PO/ReRc/S/02/CPCSEA.

Experimental Animals and Institutional Animal Ethical Clearance

4 female Wistar rats which are (8-10-week-old) (150- 180g body weight) were procured from Biogen Laboratory Animal Facility, Bengaluru. Animals were kept in sanitized polypropylene cages lined with sterile paddy husk as bedding. The dry paddy husk was used as bedding material and was replaced every morning. They had had free access to standard food pellets and water ad libitum throughout the study period, except for the night before dosing.

Ethical clearance was obtained from the Department of Pharmacology, PES College of Pharmacy, Bengaluru as per the protocol outlined in the publication of the Committee for the Purpose of Control and Supervision of Experiments on Animal standard guidelines. (CPCSEA) and approval was obtained from the Institutional Animal Ethics Committee (IAEC) with reference no FPS/IAEC/169/2024 after presenting it in the IAEC meeting held on 22/08/2024.

Acclimatization period & Identifications

All the selected animals were kept under acclimatization for 7 days before dosing. The animals were marked with a saturated picric acid solution in water for proper identification. The marking is as follows:

Table 6: Markings of Animals

Animal number	Markings
1.	No marks
2.	Head
3.	Body
4.	Tail

Dose Preparation and Schedule

The test drug was made into suspension in water with a suitable concentration. All the animals were dosed with constant dose volume i.e., 175mg/kg, 550mg/kg, 2000mg/kg.

Schedule: single dose per animal. The test drug was administered through the oral route at different dose levels to respective animals through oral gavage.

Administration of Dose

The dose was calculated according to the body weight, rat no1 is treated as normal control. The sample (dose 175mg/kg body weight) was administered orally to Rat no 2 & 3 using an oral gavage needle (not more than 2ml/100mg). The mortality was observed for 30 minutes, as there was no mortality within 30 minutes after administration

again sample dose of 550mg/kg body wt. was administered. Mortality was observed again for 30 minutes, as animals had survived a dose of 2000mg/kg body wt. After the administration of the test drug, feed was withheld for a further period of 3-4hrs.

OBSERVATION

Wellness parameters: Animals were observed continuously during the first 30 min after dosing and observed periodically (4h, 24h, 1st week, and 2nd week). Changes in wellness parameters were compared with that of the control animal (rat no 1). Individual rat weight was measured on the 1st day (before administration), 7th, and 14th day.

Table 7: Wellness parameters

Observation	30 min				4h				24h			
	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
Skin and fur	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Mucous membrane	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
Lethargy	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Coma	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Convulsion	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Tremors	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Diarrhoea	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Mortality	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF

Table 8: Wellness parameters

Observation	48hr				1 st week				2 nd week			
	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
Skin and fur	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Mucous membrane	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
Lethargy	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Coma	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Convulsion	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Tremors	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Diarrhoea	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Mortality	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF

N - Normal, NF- Not Found

Results of Acute Oral Toxicity Study

No mortality was observed on administration of *Purandara vati*. Wellness parameters and body weight analysis - showed no signs of toxicity.

DISCUSSION

Purandara vati is a herbomineral formulation which is explained in *Rasachandamshu*, beneficial in *Shwasa* and *Kasa roga*. The physico-chemical analysis is essential for validating the therapeutic potential of drugs and studying how well the active component of herbomineral drugs are absorbed and utilized in the body. pH of *Purandara vati* was 4.13, showing it is a weak acid compound. Weak acids are substances that partially ionize in solution, releasing fewer hydrogen ions (H⁺) compared to strong acids. They are usually absorbed in the highly acidic stomach (pH 1-3) by increasing the solubility of a weak acid^[7]. Since

Purandara vati is a weak acid, it can be easily absorbed in the stomach facilitating the breaking down formulation, releasing the active ingredients for absorption. *Purandara vati* had a loss on drying of 10.08% which implies a shorter life span of drug. Total ash of *Purandara vati* was 4.32% means that 4.32% of the original sample weight is composed of inorganic substances that do not combust or vaporize during the incineration process. For *Purandara Vati* acid insoluble ash was 2.36% indicating that 2.36% of the total ash content of a sample is insoluble in a specific acid solution. These impurities might include oxides, silicates, or other compounds that are not readily soluble in the acid used. The water-soluble ash of *Purandara vati* was 2.66%. This means that only a small portion of the inorganic residue is remaining after incineration, which can be dissolved by water.

These impurities might include salts, chlorides, sulphates, or other water-soluble compounds.

In XRD of *Purandara vati* 8 peaks of mercury sulphide were identified which have a cubic crystal system. Due to similar chemical composition, there might be a peak overlap between the various forms of mercury sulphide which might have caused the identification of compound as Mercury sulphide not Metacinnabar, Cinnabar or Hyper cinnabar. *Purandara vati* showed peaks as 26.42, 30.54, 43.74 which was compared with standard 2θ value. Peaks showed the presence of Mercury sulphide in cubic crystal structure. In SEM-EDX Analysis *Purandara Vati* showed 55.16% of carbon, 30.56% of oxygen, 5.52% of sulphur, and 7.21% of Hg. The data presented suggests that carbon and oxygen are key elements of the sample, indicating that it is an organic compound that includes oxides or hydroxides. In FTIR Analysis the various functional groups present in *Purandara vati* were aliphatic primary amine, carboxylic acid, alcohol, alkanes, ester, amine, sulphone, fluoro compounds, etc. In some functional groups, bonds were stronger which implies those functional groups require higher energy infrared radiations to vibrate compared to those with weaker bonds. The intensity of a peak in FTIR is proportional to the concentration of the corresponding functional group in the sample. *Aja kshira* which is used as a *Bhavana dravya* in this study is the major cause of the presence of ketones, amines, carboxylic acids and alcohols. The presence of Alkanes in the sample might be due to *Triphala churna* which is a major ingredient of formulation.

The administration of *Purandara Vati* showed no impact on the behavioural and other parameters assessed during the acute oral toxicity study in female rats. At doses up to 2000 mg/kg, there were no signs or symptoms of toxicity or mortality, indicating that the LD50 value may exceed 2000mg/kg when administered orally.

CONCLUSION

Purandara vati is a herbomineral formulation which is a weak acidic compound, might contains

silicates and oxides, have a cubic crystal system. Various functional groups like carboxylic acid, ketones and amines are present in the drug which could aid in the therapeutic action. Major elements present in *Purandara vati* are 55.16% of carbon, 30.56% of oxygen which implies that the formulation is an organic compound. Results of Acute oral toxicity showed no signs of toxicity up to a level of 2000mg/kg emphasizing the fact LD 50 of *Purandara vati* is above 2000mg/kg.

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